DOI: 10.21608/ADJC.2025.343698.1678 Advanced Dental Journal
Volume 7 (2025) | Issue 2| Pages 228- 238 ©Faculty of Dentistry-Cairo University

Original Article

Antibacterial Effect of Curcumin Nanoparticles Intracanal Medicament on 21-Days Old Enterococcus Faecalis Biofilm: An In-Vitro Study

Ebtesam Osama Abo El-Mal[1], Sara Seleem Mahmoud Seleem[2], Ahmed Maged Negm[3]

[1] Restorative Dental Sciences Department, Endodontic Division, Faculty of Dentistry, Ahram Canadian University
[2] Basic Sciences Department, Microbiology Division, Faculty of Dentistry, Ahram Canadian University
[3] Endodontic Department, Faculty of Dentistry, New Giza University

Email:ahmed.maged@ngu.edu.eg

Submitted: 9-1-2025
Accepted: 1-2-2025

Abstract

Aim: This study aimed to compare the antibacterial effect of curcumin nanoparticles intracanal medicament with nano calcium hydroxide on a 21-days old Enterococcal Faecalis (*E. faecalis)* biofilm.
Subjects and methods: A total of thirty extracted single-rooted human teeth were selected for this study. Cleaning and shaping were completed using ProTaper Next rotary system (Dentsply, Sirona) 1mm shorter from the apical foramen to size #X4 using 2.5% NaOCl irrigating solution. At the end of mechanical preparation, all canals received 5ml of 17% EDTA solution followed by 5ml of 2.5% NaOCl and finally 5ml of distilled water. Apical foramina were closed using cyanoacrylate glue and outer root surfaces were coated with double layers of nail polish. Each root canal was inoculated with 10 µl of *E. faecalis* suspension in microtubes and incubated at 37 °C for 21 days. Bacterial biofilm development was verified by scanning electron microscopic examination on the 21st day. Samples were divided into four groups. Group I 3% curcumin nanoparticle paste, Group II 5% curcumin nanoparticle paste, Group III nano calcium hydroxide and Group IV teeth were used as a negative control. The antibacterial agents were washed from the root canals after one week using 20 ml sterile saline solution and dried with paper points then debris were collected using gates glidden drills size 5. The collected debris were transformed into separate sterile Eppendorf's containing 1 mL of sterile saline and vortexed for 30 s to determine the number of E. Faecalis colonies formed.
Results: showed non-significant differences between the first three groups.
Conclusion: Curcumin nanoparticles are an effective antibacterial intracanal medicament against E. faecalis. None of the used intracanal medication totally eliminated E faecalis.
Keywords: Curcumin, Culture, Nanoparticles, *E. faecalis'*

I. INTRODUCTION

Bacteria is the primary cause of dental caries, which in turn lead to the progression of pulp and periapical disorders **(Farasat, M. and Roza, H. 2018)**. E. faecalis, which frequently manifests as biofilms, is most frequently collected from teeth with root fillings. Enterococci are gram-positive cocci that may appear individually, in pairs, or in short chains. As facultative anaerobes, they can grow both with or without oxygen **(Charles H.S et al 2006)**.

E. faecalis has certain virulence factors which enable the adherence to host cells and change the host response. Lytic enzymes, cytolysin, pheromones and lipoteichoic acid are some of these virulence agents. According to studies, E. faecalis' capacity to build a biofilm explains why it is resistant to intracanal medications for longer than 10 days if high pH cannot be maintained **(Samiei M et al 2016)**. Biofilm is composed of an extracellular polymeric matrix that shields bacteria from nutrient shortages, as well as from high alkaline environments and elevated salt concentrations caused by intracanal medicaments. **(Nelakantan P et al 2015)**

Innovative antimicrobial delivery technologies, like nanoparticles, have been developed to enhance antibacterial drugs properties during root canal treatment **(Kishen A et al 2008)**. Particles having exterior diameters of 1 to 100 nm are referred to as nanomaterials because of their small size, high surface-to-volume mass ratio, and elevated chemical reactivity. Due to their increased surface area and charge density, nanoparticles can interact more effectively with the negatively charged surfaces of bacterial cells, leading to improved antibacterial activity. As a result, they have been widely utilized in various medical fields **(Silver S et al 2006).**

Curcumin, a plant-derived compound from turmeric root, is known for its antimicrobial and anti-inflammatory properties. It also exhibits antioxidant and anti-cancer effects, making it clinically significant for preventing and treating various diseases **(Kocaadam B and Sanlier 2017)**. When used as an intracanal irrigant during endodontic procedures, curcumin demonstrated effective disinfection, likely due to its ability to permeabilize and damage bacterial membranes. Additionally, curcumin has been utilized in the creation of electrospun fibers for biomedical applications, such as skin tissue regeneration **(Mouthuy PA et al 2017)**.

The purpose of this study was to compare the antibacterial activity of nano-calcium hydroxide paste intracanal medication with curcumin nanoparticles at varying concentrations (3% and 5%) on a 21-day-old E. faecalis biofilm. According to the null hypothesis, the tested materials will not differ from one another.

II. SUBJECTS AND METHODS

The current study has been approved by the Institutional Review Board of the Faculty of Oral & Dental Medicine, Ahram Canadian University (IORG0010868, IRB000012891#57). The sample size was determined using Prabhakar's prior research **(Prabhakar AR et al 2013)**, using an independent t-test and G*Power 3.1.9.7. With the control group having a mean ± standard deviation of 285.4 ± 19, and the intervention group a mean ± standard deviation of 328.4 ± 23, the study required a minimum of 6 subjects per group (18 subjects across three groups). To account for a 20% dropout rate, the sample size was increased to 8 subjects per group (24 subjects total).

Selection of teeth:

A total of thirty extracted single-rooted human teeth were selected for this study. The inclusion criteria were single, straight-rooted teeth with mature apices. Teeth with cracks, fractured roots, resorptions, calcified canals, or those previously treated with root canal therapy were excluded **(Yadav RK et al 2018)**. Ultrasonic scaler was used to remove any calculus or soft tissue debris, then samples were kept in normal saline solution. A tapered diamond stone set on a high-speed contra-angle handpiece with water cooling was used to section the anatomical crowns at the cemento-enamel junction. A standard root length of 14 mm ± 1 mm was used **(Prabhakar AR et al 2013).**

Preparation of roots:

A Manual K-file size #15 (Mani, Inc., Japan) was used to determine the working length of each root by inserting the file into the root apex until the tip was visible at the apical foramen, then retracting it by 1 mm **(Prabhakar AR et al 2013)**. Cleaning and shaping were performed using the ProTaper Next rotary system (Dentsply Maillefer, Ballaigues, Switzerland)

up to an apical size of #X4, with 2.5% NaOCl as the irrigating solution and EDTA cream (MD ChelCream, Meta BioMed, Korea) as the lubricant. After mechanical preparation, the canals were irrigated with 5 ml of 17% EDTA solution, followed by 5 ml of 2.5% NaOCl, and concluded with 5 ml of distilled water as the final irrigation sequence **(Yadav RK et al 2018).** Following the preparation, the apical foramina of all specimens were sealed with cyanoacrylate glue to prevent bacterial microleakage **(Selvi MM et al 2022)**. The outer surfaces of the roots, including the apical foramina, were coated with two layers of nail polish, leaving the orifices exposed.

Each root was individually placed in an Eppendorf tube and sealed in a sterilization pouch, then autoclaved at 132°C for 30 minutes at 27.1 PSI to eliminate microorganisms from the teeth **(Prabhakar AR et al 2013, Saber SELD and El-Hady SA 2012)**.

Classification of samples:

Samples were randomly divided into five groups **(Yadav RK et al 2018):**

Group I: 3% Curcumin nanoparticle paste (Nanogate company, Cairo, Egypt) intracanal medicament (n=8 samples).

Group II: 5% Curcumin nanoparticle paste (Nanogate company, Cairo, Egypt) intracanal medicament (n=8 samples).

Group III: Nano calcium hydroxide intracanal medicament paste (Nanogate company, Cairo, Egypt) (n=8 samples).

Group IV: 3 teeth were used as negative control (inoculation of E.faecalis without placement of intracanal medicament)

Group V: 3 samples were viewed under the scanning electron microscope (SEM) to confirm biofilm formation.

Curcumin nanoparticle pastes (Nanogate company, Cairo, Egypt) concentrations of 3% and 5% were prepared according to **(Sometil et al. 2019)** who introduced these concentrations as endodontic irrigating solutions.

Cultivation and inoculation of *E. faecalis* biofilm

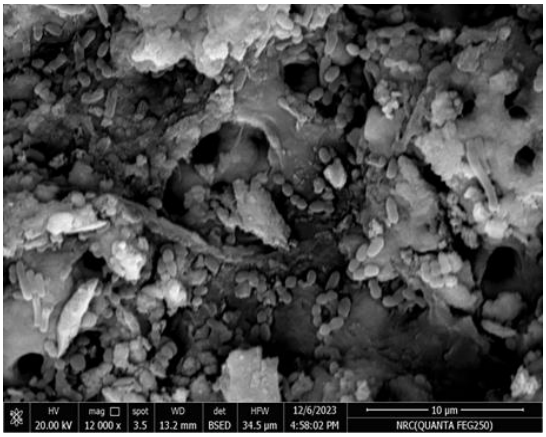
E. faecalis (ATCC 29212) was cultured on a blood agar plate and incubated at 37°C for 24 hours. The resulting colonies were then suspended in 5 ml of brain heart infusion (BHI) broth and incubated for an additional 24 hours. A pure suspension of E. faecalis with a concentration of 1.5×10^8 CFU/ml was prepared using spectrophotometry, adjusting the turbidity to match a 0.5 McFarland standard. Each root canal was inoculated with 10 µl of the E. faecalis suspension in microtubes and incubated at 37°C for 21 days under aseptic aerobic conditions. The inoculation was renewed every 3 days to maintain bacterial viability **(Sometil et al. 2019).**

Verification of Biofilm development:

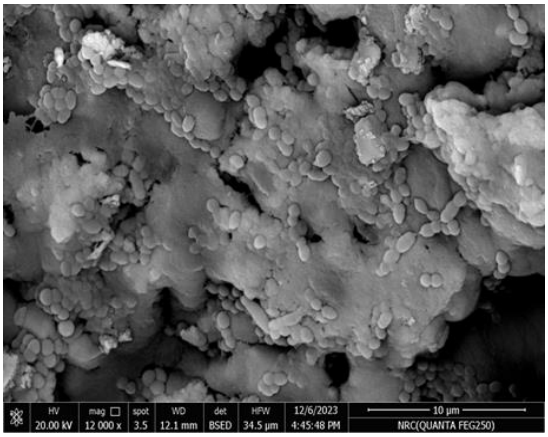
Bacterial biofilm development was verified by the scanning electron microscopic examination on the 21st day after bacterial inoculation inside the root canals. Three randomly selected samples (group V) were applied then grooves were established longitudinally through grooves along the root length then split using a hammer and chisel into two identical halves. After fixation and preparation of the six halves of the roots, the whole canals were assessed under Field Emission Scanning Electron Microscope (FESEM), Quanta FEG 250 attached by energy dispersive x-ray analysis (EDAX) model (AMETEK) at high voltage 20 kv. SEM assessment was made to ensure the formation of biofilm (fig. 1). **(Yadav RK et al 2018, Eskandarinezhad M et al 2022)**

Primary sampling:

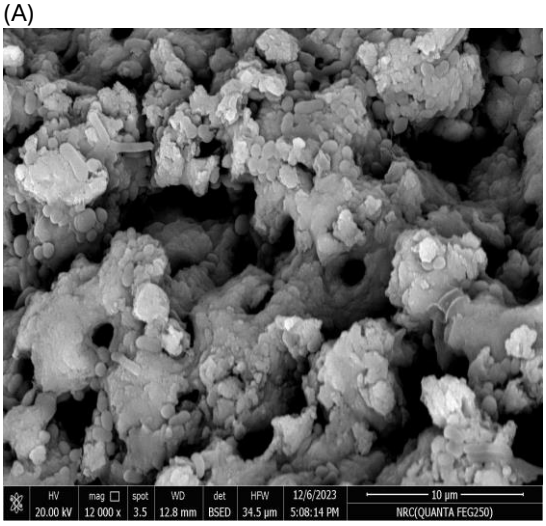
A Sterile gauze was used to carefully extract the teeth from the microtubes, and a sterile 30-gauge needle was used to aspirate the BHI broth out of the root canal system. The canals were then vortexed for 30 seconds and flushed with 3 mL of sterile saline for 30 seconds. After 30 seconds of inserting a paper point into the root canal, the tip was moved to a sterile microtube with 1 mL of saline and vortexed for an additional 30 seconds. Using the spread plate approach, 100 µL of each 10-fold serial dilution up to 1:100 was plated onto a BHI agar plate. Colonies were counted after a day, and the dilution factor was used to compute the results (fig. 2). **(Shaaban S et al 2023)**



(A)



(B)



(C)

Fig.1*: SEM photos of 21-days old E.Faecalis biofilm on the walls of the root canals: (A) at the apical part of the root canal, (B) at the middle part of the root canal, (C) at the coronal part of the root canal*

Insertion of the intracanal medicaments:

After exclusion of three samples as negative control groups, the remaining 24 samples were randomly divided into three groups (n=8/group) according to the intracanal medicament injected into the canals as previously mentioned. The medicaments were injected by meaning of disposable tips provided by Meta BioMed to cover the entire length of the canal. **(Eskandarinezhad M et al 2022)** The orifices were finally sealed with composite resin restoration material.

To allow expression of the antimicrobial properties for the canal medicaments under clinical conditions, samples were incubated for one week at 37ºC and 100% humidity **(Saber SELD and El-Hady SA 2012).**

Preparation of specimens for antimicrobial analysis:

Using disposable plastic syringes and 20 mL of sterile saline solution, the antibacterial agents were rinsed out of the root canals after a week **(Yadav RK et al 2018)**. After using sterile paper points to dry the canals, debris were removed from the entire root canal length using #5 Gates Glidden Drills (Mani, Tochigi, Japan). To determine the number of E. faecalis colonies, the collected debris were vortexed for 30 seconds after being moved into several sterile Eppendorf tubes with 1 mL of sterile saline. All the previously mentioned steps were done in the following operations (fig.2). **(Eskandarinezhad M et al 2022)**

Statistical analysis

Minitab® statistical software version 16, Microsoft Excel® 2016, and the Statistical Package for the Social Sciences (SPSS)® version 24 were employed to gather, arrange, and statistically analyse the data. The Shapiro-Wilk test and the Kolmogorov-Smirnov test were employed to evaluate the data's normality, and both tests showed that the data had a normal distribution. Therefore, a One-Way ANOVA test was used to compare groups, and Tukey's Post Hoc test was used for multiple comparisons. A paired t-test was employed to compare the data before and after therapy. A level of significance of $p < 0.05$ was established.

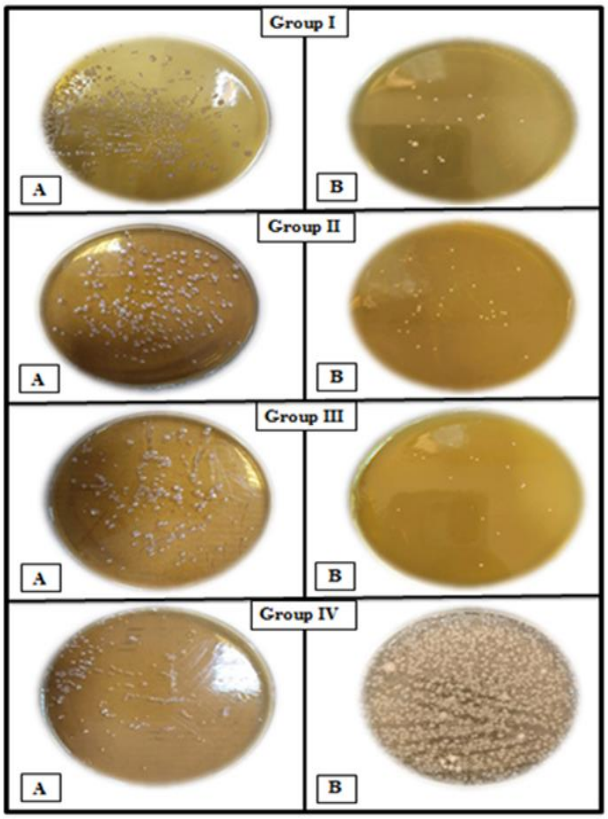


Fig.2: *Colony Forming Unit assay. (Group I) represents E. faecalis treated with 3% Curcumin nanoparticle paste intracanal medicament. (Group II) represents E. faecalis treated with 5% Curcumin nanoparticle paste intracanal medicament. (Group III) represents E. faecalis treated with Nano Calcium Hydroxide intracanal medicament paste. (A) in all groups represents E. faecalis before treatment and (B) represents E. faecalis after treatment. (Group IV) negative control group where (A) was after immediate inoculation and (B) after 21 days.*

III. RESULTS

Evaluation Enterococcus faecalis CFU (x 10^5):

Intragroup comparison:

Comparison between CFU before and after treatment was performed and demonstrated that there was a significant change in CFU in all groups as:

In group I: there was a significant decrease from (199.5 ± 87.72) before treatment to (16 ± 22.67) after treatment with (183.5 ± 73.23) difference between them as P=0.0001.

In group II: there was a significant decrease from (182.38 ± 78.91) before treatment to (12.88 ± 13.34) after treatment with (169.5 ± 77.14) difference between them as P=0.0001.

In group III: there was a significant decrease from (154.5 ± 82.1) before treatment to (10.5 ± 16.85) after treatment with (144.0 ± 71.61) difference between them as P=0.0001.

Table (1): Descriptive results of CFU (x 10^5) before and after treatment in all groups, comparison between before and after (Intragroup comparison) using Paired t test:

						Paired Differences				
		Minimum	Maximum	Mean	Standard Deviation	Mean	Std. Deviation	Std. Error Mean	95% CI of the Difference	
									Lower	Upper
Group I	Before	74.00	300.00	199.50	87.72	-183.50	73.23	25.89	-244.72	-122.28
	After	0.00	57.00	16.00	22.67					
Group II	Before	92.00	298.00	182.38	78.91	-169.50	77.14	27.27	-233.99	-105.01
	After	0.00	38.00	12.88	13.34					
Group III	Before	55.00	260.00	154.50	82.10	-144.00	71.61	25.32	-203.87	-84.13
	After	0.00	50.00	10.50	16.85					

M: mean ***SD: standard deviation***

****Significant difference as P<0.05.***

Table (2): Means and standard deviations of CFU before treatment, after treatment, and difference between before and after treatment in all groups, comparison between all groups using One Way ANOVA test followed by Tukey`s Post Hoc test for multiple comparisons:

	Group I		Group II		Group III		P value
	M	SD	M	SD	M	SD	
Before	199.50	87.72	182.38	78.91	154.50	82.10	0.55
After	16.00	22.67	12.88	13.34	10.50	16.85	0.83
Difference	-183.50	73.23	-169.50	77.14	-144.00	71.61	0.56
% of decrease	84.00	22.67	87.13	13.34	89.50	16.85	0.81

M: mean ***SD: standard deviation*** ****Significant difference as P<0.05.***

Means with different superscript letters were significantly different as P<0.05.

Means with the same superscript letters were insignificantly different as P>0.05.

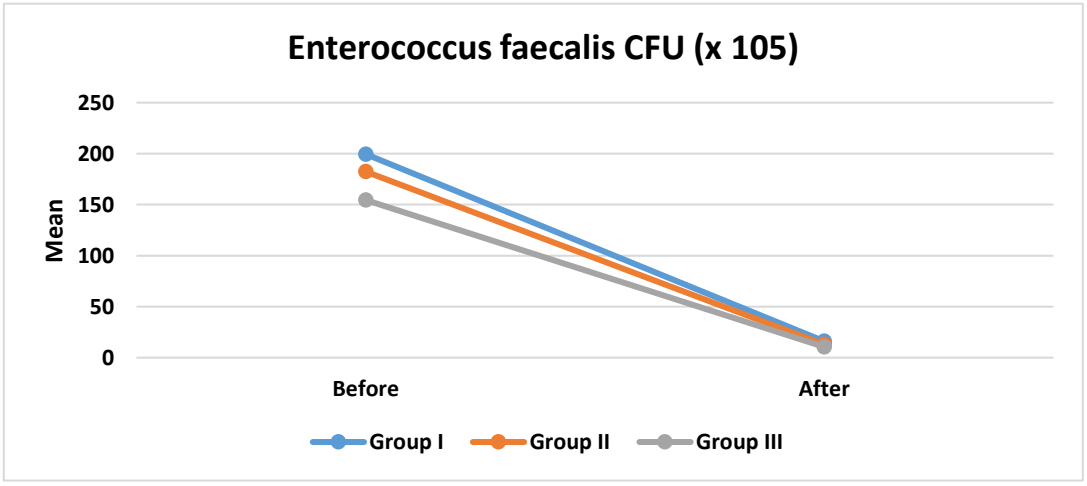


Fig. 3: *Line chart representing CFU before and after treatment in all groups*

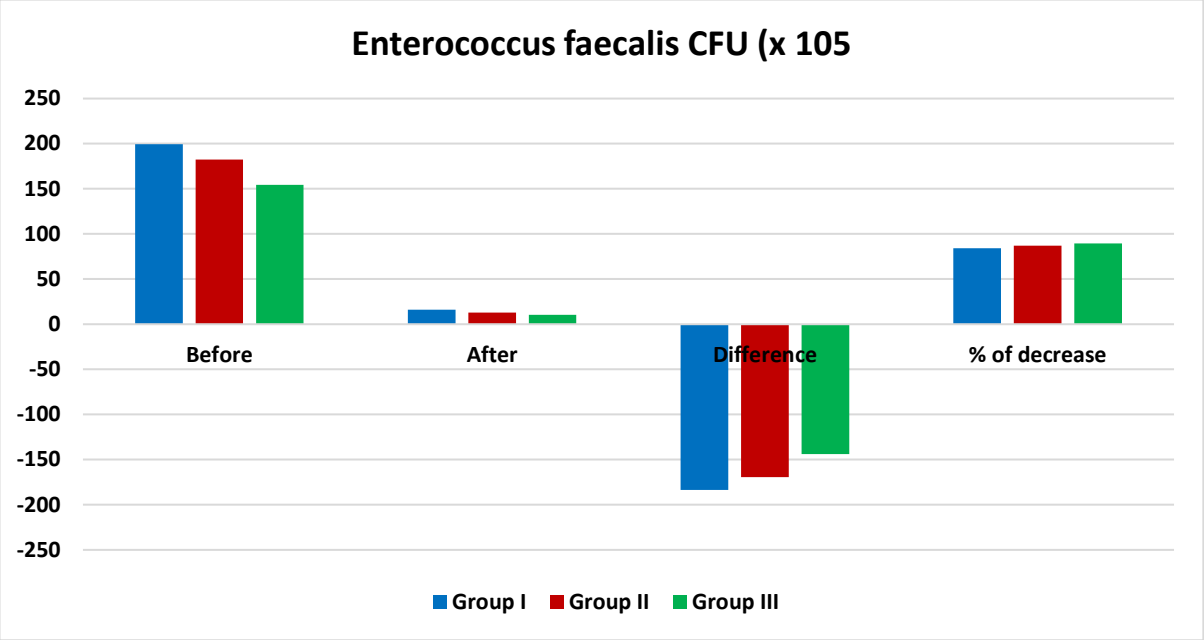


Fig. 4: *Bar chart representing CFU before treatment, after treatment, difference between before and after in all groups.*

Intergroup comparison:

Comparison between different groups was performed and demonstrated insignificant difference between them regarding CFU before treatment (P=0.55). After treatment (0.83), difference between before and after (0.56), and percentage of decrease (0.81). (Table 2, fig 4).

IV. DISCUSSION

Root canal biofilm is a complex structure composed of polysaccharides, proteins, and microbial cells, making it notoriously difficult to eliminate and highly resistant to conventional treatments. Nanoparticle-based antimicrobial agents have been suggested to be used as a potential solution for intracanal infections due to their strong ability to disrupt biofilms and prevent bacterial adhesion to root canal dentine **(Kishen A 2010).**

The limitations of current intracanal medicaments have sparked interest in finding more effective, safe, and potent herbal alternatives, such as curcumin, for root canal disinfection. Herbal agents like curcumin provide several advantages, including easy availability, minimal adverse effects, low cost, better patient tolerance, and superior antibacterial properties **(Digole VR et al 2020).** As both an anti-inflammatory and antibacterial agent, curcumin exhibited no toxic effects on the human body, even at high doses **(Fani M and Kohanteb J 2012)**. While the exact mechanism of action remains unclear, curcumin is thought to inhibit the aggregation of protofilaments, and hence prevents bacterial cell proliferation **(Svensäter G and Bergenholtz G 2004).**

Curcumin has also provided antibiofilm activity against a variety of bacterial species, including *A. baumannii*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *P. mirabilis*, *S. marcescens*, *S. epidermidis*, and *S. mutans* **(Stojicic S et al 2013).**

The aim of the current study was to compare the antibacterial effectiveness of Curcumin nanoparticle paste with nano calcium hydroxide intracanal medicament paste. *E. faecalis* is known for its high resistance to endodontic treatments, partly due to its tendency to penetrate dentinal tubules, tolerance to high alkalinity, elevated salt concentrations, and starvation conditions. Additionally, *E. faecalis* has the capability to form biofilms **(Cogulu D et al 2007),** further complicating treatment efforts.

In the current study, the biofilm was used being more common in persistent infections and 1000 times more resistant to antibacterial agents **(Moghadamtousi, S.Z et al 2014)**. The microbiological procedures have been carried out in a laminar air flow to avoid

contamination. Since saline has no antibacterial properties, it was used to remove the medications from root canals with no influence the results of study **(Raorane CJ et al 2019).**

The presence of *E. faecalis* in root canals can be verified by using either culture methods or molecular techniques. According to **(Cogulu et al. 2007),** both methods are sufficiently sensitive to identify *E. faecalis* in both permanent and deciduous teeth. In the present study, the culturing technique was employed. Culture techniques are useful as an initial approach for identifying dominant bacterial species or correlating specific bacteria with particular clinical outcomes. However, molecular techniques offer the advantage of detecting bacteria that are difficult or impossible to culture, as well as providing more detailed analysis of bacterial behaviour. Despite these advantages, results obtained from in vitro tests should be studied cautiously, as they may not be able to fully reflect the clinical effectiveness of the agents being tested.

To assess the antibacterial effect of the medications, colony-forming units (CFU/ml) were calculated. While many previous studies have employed the agar disk diffusion method to evaluate antimicrobial activity, this method is less precise. On the contrary, counting CFUs provides a more accurate assessment of the viable bacteria present in the root canals **(Sharma G et al 2014).**

In the present study all groups demonstrated a significant decrease in CFU after treatment (P=0.0001) except the negative control group which recorded a significant increase in CFU (P=0.0001), and hence the null hypothesis was accepted. The decrease in the CFU was insignificant in the groups treated with 3% curcumin nanoparticles, 5% curcumin nanoparticles and nano calcium hydroxide paste. Accordingly, curcumin nanoparticles paste proved to be an effective intracanal medicament. This may be attributed to the fact that curcumin inhibits the cytokinesis of *E. faecalis* by mobilizing filamentation, which is an antibacterial action. Additionally, it inhibited the destruction of the peptidoglycan cell wall of bacteria, as well as the cytokinetic Z-ring genesis in bacteria **(Li X et al 2019).**

Calcium hydroxide exerts its antibacterial effects by damaging the bacterial cell wall and creating a highly alkaline environment which denatures proteins, leading to cell death. However, the buffering capacity of dentin partially inhibits its effectiveness. Additionally, *E. faecalis* can survive in the presence of calcium hydroxide due to its proton pump, which helps maintain pH balance **(Jhamb S et al 2010).**

Our findings are in harmony with those of **(Vasudeva A et al 2017)** and **(Prabhakar AR et al 2013)**, who demonstrated that *Curcuma longa* has antibacterial activity against *Enterococcus faecalis*. Furthermore, **(Neelakantan P et al 2011)** showed that curcumin effectively eradicates *E. faecalis* biofilm after 2 days and 2 weeks of treatment. Similarly, **(Tyagi et al. 2015)** reported that curcumin exhibited strong antibacterial effects against *Pseudomonas aeruginosa*, *Escherichia coli*, *E. faecalis*, and *Staphylococcus aureus*. Additionally, **(Eskandarinezhad et al. 2022**) found that curcumin's effect on *E. faecalis* biofilm was superior to that of calcium hydroxide. **(Mandroli P and Bhat K 2013)** also concluded that curcumin demonstrated antibacterial potential against common endodontic bacterial strains.

In contrast, **(Yadav RK et al. 2018)** found that calcium hydroxide intracanal medication could not effectively eliminate *E. faecalis*, which contradicts our results which indicated that calcium hydroxide can reduce the CFU count of *E. faecalis*.

However, a study by **(Swapnil SM et al. 2017)** reported that curcumin passes a lower antibacterial effect compared to Triphala and calcium hydroxide against planktonic *E. faecalis*. This discrepancy may be attributed to the differences in experimental methodology, as the Swapnil et al. study used the agar disk diffusion test, while our study utilized the colony-forming unit method. Additionally, the microorganisms used in their study were not in a biofilm state, unlike in our study.

V. CONCLUSION

- Curcumin nanoparticle is effective antibacterial intracanal medicament against *E. faecalis.*
- None of the used intracanal medication totally eliminated E faecalis.
- Herbal antibacterial agents are strong substitutes to calcium hydroxide intracanal medication in terms of lack of adverse effect and better patient tolerance.

Conflict of Interest:

The authors declare no conflict of interest.

Funding:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics:

This study protocol was approved by the ethical committee of the faculty of oral and dental medicine Ahram Canadian University on: 1/6/2023, Research number: IRB00012891#57.

Data availability statement:

Data only from published papers.

Authors contribution statement:

Ebtesam Osama Abo El-Mal: Study design, data collection, methodology and writing.

Sara Seleem Mahmoud Seleem: Study design, bacterial lab work, writing and data analysis.

Ahmed Maged Negm: Review, study design, methodology, data collection, writing and revision.

VI. REFERENCES

1. Charles, H.S., Scott, A.S., Thomas, J.B. and Owatz, C.B. (2006) 'Enterococcus faecalis: Its role in root canal treatment failure and current concepts in retreatment', *Journal of Endodontics*, 32, pp. 93–98.
2. Cogulu, D., Uzel, A., Oncag, O., Aksoy, S. and Eronat, C. (2007) 'Detection of Enterococcus faecalis in necrotic teeth root canals by culture and polymerase chain reaction methods', *European Journal of Dentistry*, 1, pp. 216–221.
3. Digole, V.R., Dua, P., Shergill, S., Pathak, P., Kumar, V., Prakash, P. (2020) 'Comparative evaluation of antimicrobial efficacy of calcium hydroxide, curcumin and aloe vera as an intracanal medicament: An in-vivo study', *IP Indian Journal of Conservative and Endodontics*, 5(3), pp. 114–119.
4. Eskandarinezhad, M., Barhaghi, S., Allameh, K., Sadrhaghighi, A., & Katebi, K. (2022) 'The comparison of calcium hydroxide, curcumin, and Aloe vera antibacterial effects on 6-week-old Enterococcus faecalis biofilm as an intracanal medicament: An in vitro study', *Dental Research Journal (Isfahan)*, 19, pp. 14-19.
5. Fani, M. and Kohanteb, J. (2012) 'Inhibitory activity of Aloe vera gel on some clinically isolated cariogenic and periodontopathic bacteria', *Journal of Oral Science*, 54, pp. 15–21.
6. Farasat, M. and Roza, H. (2018) 'Evaluation of antimicrobial efficacy of nanosilver solution, sodium hypochlorite and normal saline in root canal irrigation of primary teeth', *Contemporary Clinical Dentistry*, 9, pp. S227–S232.
7. Jhamb, S., Nikhil, V. and Singh, V. (2010) 'An in vitro study of antibacterial effect of calcium hydroxide and chlorhexidine on Enterococcus faecalis', *Indian Journal of Dental Research*, 21, pp. 512–514.
8. Kishen, A. (2010) 'Advanced therapeutic options for endodontic biofilms', *Endodontic Topics*, 22, pp. 99–123.
9. Kishen, A., Shi, Z., Shrestha, A. and Neoh, K.G. (2008) 'An investigation on the antibacterial and antibiofilm efficacy of cationic nanoparticulates for root canal disinfection', *Journal of Endodontics*, 34, pp. 1515–1520.
10. Kocaadam, B. and Sanlier, N. (2017) 'Curcumin, an active component of turmeric (Curcuma longa), and its effects on health', *Critical Reviews in Food Science and Nutrition*, 57, pp. 2889–2895.
11. Li, X., Yin, L., Ramage, G., Li, B., Tao, Y., Zhi, Q., Lin, H. and Zhou, Y. (2019) 'Assessing the impact of curcumin on dual-species biofilms formed by *Streptococcus mutans* and *Candida albicans*', *Microbiology Open*, 8, e937.
12. Mandroli, P., & Bhat, K. (2013) 'An in-vitro evaluation of antibacterial activity of curcumin against common endodontic bacteria', *Journal of Advanced Pharmaceutical Sciences*, 3, pp. 106–108.
13. Moghadamtousi, S.Z., Kadir, H.A., Hassandarvish, P., Tajik, H., Abubakar, S. and Zandi, K. (2014) 'A review on antibacterial, antiviral, and antifungal activity of curcumin', *Biomed Research International*, 186864.

14. Mouthuy, P.A., Somogyi Skoc, M., Cipak Gasparovic, A. et al. (2017) 'Investigating the use of curcumin-loaded electrospun filaments for soft tissue repair applications', *International Journal of Nanomedicine*, 12, pp. 3977–3991.
15. Neelakantan, P., Cheng, C.Q., Moharaj, R., Sriaman, P., Subarao, C. and Sharma, S. (2015) 'Antibiofilm activity of three irrigation protocols activated by ultrasonic, diode laser or Er:YAG laser in vitro', *International Endodontic Journal*, 48(6), pp. 602–610.
16. Neelakantan, P., Subbarao, C. and Subbarao, C.V. (2011) 'Analysis of antibacterial activity of curcumin against Enterococcus faecalis', *International Journal of Current Research and Review*, 3, pp. 101–104.
17. Prabhakar, A.R., Swapnil, T., Savita, H. and Sugandhan, S. (2013) 'Comparison of antibacterial efficacy of calcium hydroxide paste, 2% chlorhexidine gel and turmeric extract as an intracanal medicament and their effect on microhardness of root dentin: An in vitro study', *International Journal of Clinical Pediatric Dentistry*, 6(3), pp. 171–177.
18. Raorane, C.J., Lee, J.H., Kim, Y.G., Rajasekharan, S.K., García-Contreras, R. and Lee, J. (2019) 'Antibiofilm and antivirulence efficacies of flavonoids and curcumin against Acinetobacter baumannii', *Frontiers in Microbiology*, 10, pp. 990.
19. Saber, S.E.L-D. and El-Hady, S.A. (2012) 'Development of an intracanal mature Enterococcus faecalis biofilm and its susceptibility to some antimicrobial intracanal medications: An in vitro study', *European Journal of Dentistry*, 6(1), pp. 43–50.
20. Samiei, M., Farjami, A., Dizaj, S.M. and Lotfipour, F. (2016) 'Nanoparticles for antimicrobial purposes in endodontics: A systematic review of in vitro studies', *Materials Science and Engineering C*, 58, pp. 1269–1278.
21. Selvi, M.M., Selvabalaji, A., Chonat, A., Ananthan, V., Mani, E. and Chinnaswamy, A.D. (2022) 'A comparative evaluation of antimicrobial efficacy of triphala and calcium hydroxide as intracanal medicament: An in vitro study', *Journal of Pharmacology and Biopharmaceutical Sciences*, 14, pp. S774–S780.
22. Shaaban, S., Genena, S., Elraggal, A., Hamad, G.M., Meheissen, M.A., Moussa, S. (2023) 'Antibacterial effectiveness of multi-strain probiotics supernatants intracanal medication on Enterococcus faecalis biofilm in a tooth model', *BMC Oral Health*, 23(1), pp. 1–11.
23. Sharma, G., Raturi, K., Dang, S., Gupta, S. and Gabrani, R. (2014) 'Combinatorial antimicrobial effect of curcumin with selected phytochemicals on *Staphylococcus epidermidis*', *Journal of Asian Natural Products Research*, 16, pp. 535–541.
24. Silver, S., Le Phung, T. and Silver, G. (2006) 'Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds', *Journal of Industrial Microbiology and Biotechnology*, 33, pp. 627–634.
25. Sotomil, J.M., Münchow, E.A., Pankajakshan, D., Spolnik, K.J., Ferreira, J.A., Gregory, R.L. and Bottino, M.C. (2019) 'Curcumin—a natural medicament for root canal disinfection: Effects of irrigation, drug release, and photoactivation', *Journal of Endodontics*, 45(11), pp. 1371–1377.
26. Stojicic, S., Shen, Y. and Haapasalo, M. (2013) 'Effect of the source of biofilm bacteria, level of biofilm maturation, and type of disinfecting agent on the susceptibility of biofilm bacteria to antibacterial agents', *Journal of Endodontics*, 39, pp. 473–477.
27. Svensäter, G. and Bergenholtz, G. (2004) 'Biofilms in endodontic infections', *Endodontic Topics*, 9, pp. 27–36.
28. Swapnil, S.M., Sharma, A., Shah, N., Mandlik, J., & Ghogare, A.A. (2017) 'Antimicrobial efficacy of triphala and curcumin extract in comparison with calcium hydroxide against *Enterococcus faecalis* as an intracanal medicament: A study', *Paripex - Indian Journal of Research*, 6, pp. 876–879.
29. Tyagi, P., Singh, M., Kumari, H., Kumari, A., & Mukhopadhyay, K. (2015) 'Bactericidal activity of curcumin I is associated with damaging of bacterial membrane', *PLOS ONE*, 10(12), e0121313.
30. Vasudeva, A., Sinha, D.J., Tyagi, S.P., Singh, N.N., Garg, P. and Upadhyay, D. (2017) 'Disinfection of dentinal tubules with 2% chlorhexidine gel, calcium

hydroxide and herbal intracanal medicaments against Enterococcus faecalis: An in-vitro study', *Singapore Dental Journal*, 38, pp. 39–44.

31. Yadav, R.K., Tikku, A.P., Chandra, A., Verma, P., Bains, R. and Bhoot, H. (2018) 'A comparative evaluation of the antimicrobial efficacy of calcium hydroxide, chlorhexidine gel, and a curcumin-based formulation against Enterococcus faecalis', *National Journal of Maxillofacial Surgery*, 9(1), pp. 52–55.